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NAME OF SUPERVISOR/NOM DU DIRECTEUR DE THÈSE Dr. H. Wenger

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LIVER GLYCOGEN REPLETION PATTERN FOLLOWING
SPRINT AND ENDURANCE EXERCISE IN MALE RATS

by



JANE M. GARDNER

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Liver Glycogen Repletion Pattern Following Sprint and Endurance Exercise in Male Rats submitted by Jane M. Gardner in partial fulfilment of the requirements for the degree of Master of Science.

Howard H. Williams

Supervisor

Jo Russell

Quinn

Date... *June 7* 1978

ABSTRACT

Fifty-five male Wistar rats were equally divided into sprint and endurance groups. Each group was trained and conditioned for 15 days to enable the animals to meet the criteria for their respective exercise tests.

Prior to the administration of the exercise test each of the two groups was divided into equal subgroups corresponding to the time of sacrifice following the exercise test. These groupings were Pre, 0, 2, 6, 12, 18, 24, and 48 hours. The exercise test for the endurance group consisted of one hour of continuous running at 30 m/min (10 percent incline). The exercise test criteria of the sprint group constituted intermittent running (10 seconds work to 30 seconds rest) at 90 m/min 10 percent incline for one hour and 20 minutes. The animals were then sacrificed according to their respective subgroups, and the excised liver frozen with isopentane cooled by liquid nitrogen. Analysis of the liver glycogen content was done by the anthrone method of Seifter et al. (1950).

Glycogen depletions were greater with the sprint group than the endurance group. It was found that pre-exercise glycogen values were attained at 6, 24 and 48 hours following heavy exercise. Also, the two different types of acute exercise used did not differentially affect liver glycogen repletion.

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SECTION I

INTRODUCTION

Man's fascination with himself and his interaction with his environment have been the underlying motivating factors in the study of the sciences. He has attacked scientific dilemmas from many aspects. By studying animals and plants at all levels, he hopes to find an understanding of "the nature of things", and thus discern his position in this order.

Exercise physiologists have placed their emphasis on man's internal responses with the exercise environment. Where direct study of man is not feasible, animals such as rats have become the experimental model. Through the examination of the effects of exercise, it has become an accepted fact that there are many parameters of metabolism and circulation which are altered acutely and chronically in response to exercise. Histochemical, biochemical and surgical techniques for tissue analysis and biopsies provide the most recent means for cellular and organ research.

Ultimately, physical work capacity of muscle is limited by the amount of contractile (myofibrillar) protein in the skeletal muscle (Jaweed et al., 1974) and/or the energy producing ability of the muscle to sustain contractions (Wenger and Reed, 1976; Simonson, 1971; Gollnick

and King, 1969).

Enhanced metabolic capacity for energy production is directly attributable to such things as changes in hormone levels (Goodman, 1974; Hartley et al., 1972; White, 1973) and in the activity and concentrations of intermediary metabolic enzymes (Bass et al., 1969; Staudte and Pette, 1972; Saltin, 1973; Holloszy, 1975) with exercise overloads.

These energy needs are met by energy stores found in two areas -- the muscle itself and the liver. Glycogen or glucose is the known substrate for energy production in exercise in which work intensity is greater than that which is accomplished by oxidative means (Saltin, 1973).

With anaerobic exercise, the muscle readily uses its limited supply of ATP (Gollnick and King, 1969), but as activity continues, the source of ATP becomes the anaerobic hydrolysis of glycogen to lactate. With repeated bouts of high intensity exercise, the muscle glycogen can be severely depleted (Gollnick and Hermansen, 1973). Although prolonged, low intensity power outputs utilize primarily fat as a fuel, some muscle glycogen is always catabolized. Hence muscle glycogens can also be severely depleted in response to low intensity prolonged work efforts. With the substantial depletion of glycogen stores in the active muscle, additional carbohydrate fuel is required and the blood is the most available source. Then, in response to decreased blood glucose level and the subsequent increases in glucogenic hormones, the liver glycogen is utilized to

augment the lower blood glucose levels. It is then transported to the active muscle to serve as the carbohydrate fuel to meet further energy demands.

It should be noted that glycogen reserves of non-working muscles cannot be transferred to the working ones (Gollnick and King, 1969). This is due to the fact that muscle does not contain glucose-6-phosphatase and hence cannot release glucose from glycogen back into circulation (Gollnick and King, 1969, p. 26). Only liver has this capability.

Thus there is an important role played by the liver in the mobilization of glucose to augment muscle glycogen during exercise. The levels of liver glycogen prior to exercise could therefore ultimately affect the ability to perform repeated high intensity power outputs or prolonged lower power efforts. Thus, the replenishment pattern of liver glycogens following these two different types of exercise could dictate when the capacity to do another exercise bout was optimal.

Statement of the Problem

This study was designed to examine the time course and repletion pattern of liver glycogen following either sprint or endurance exercise programmes. Since total work outputs over an exercise session may affect the depletion and repletion rates (MacDougall, 1977), the sprint and endurance exercise tests were equated on total distance run.

SECTION II

METHODOLOGY

Animal Care

A total of 55 male Wistar rats were obtained at approximately four to five weeks of age (90-100 g) from Woodlyn Farms Limited, Guelph, Ontario. The animals were housed in individual, self-cleaning cages, and were fed, ad libitum, a consistent diet of Purina Laboratory Chow (23 percent crude protein, 4.5 percent crude fat; see Appendix D for detailed list of constituents) and water.

Upon admission to the laboratory, the animals were weighed and permitted five days of orientation to laboratory conditions.

Daily laboratory routine included cage rotation, replenishment of food and water, and the changing of soiled papers. Laboratory temperature and barometric pressure were monitored periodically to avoid environmental fluctuations.

Since the rat is a nocturnal animal, the animals were placed on a reversed day/night cycle whereby the dark period was from 6:00 a.m. to 6:00 p.m. All training and laboratory activity took place during this time.

Preceding each training session, the animals were examined for injuries and disease. If any cuts or abrasions

were immediately treated with Scarlet Oil Antiseptic Spray. Interference with running due to injury resulted in a one to two day rest, depending upon the severity. Bad runners or ill tempered animals were eliminated from the study (see Table 1). The attrition rate of 11-12 percent is relatively high but since this study was acute, there was little time to bring poor runners along slowly and hence they were deleted.

Training Protocol

All animals were exercised on a motor driver treadmill consisting of ten, 75 cm x 10 cm compartments. Motivation to run was provided via an electric grid located at the rear of each compartment such that a shock was received upon contact (50-70 V). The treadmill speed was calibrated prior to each training session. The sprint work to rest intervals were automatically regulated by a timing device attached to the treadmill.

In order to study the liver repletion pattern following an acute bout of either sprint or endurance exercise, animals had to be both trained and conditioned to be able to perform the exercise. Hence, the 55 animals were randomly divided into either a sprint or an endurance exercised group, and then progressively overloaded on the treadmill until they could perform their respective exercise test.

Conditioning took place four days per week: Monday, Tuesday, Thursday and Friday, with the remaining three days

being set aside as rest days. One conditioning session consisted of exercising all animals from both groups.

Both groups followed the same regimen for the first three days, to introduce them to treadmill running. The complete conditioning progression for treadmill speed is graphically displayed in Figures 1 and 2. On day one both groups ran for six minutes total; day two saw the animals running for ten minutes total, five minutes at each respective treadmill speed and day three, one minute and 14 minutes respectively.

All animals went on a maintenance programme on day 13 to ensure animal fitness whilst waiting to perform their exercise test.

A. Endurance Regimen

After day three, only the length of time running changed for the next several days. Day four saw the first progression, run for one minute with the remaining daily exercise done for 19 minutes. The running time for day five was 25 minutes; day six, 30 minutes and day seven, 40 minutes. On day eight the running time was increased to 45 minutes and so remained until day 13.

The remaining two days were all animal maintenance days where the speed remained the same but the time running was reduced to 30 minutes. Figure 1 gives a detailed description of the speed and grade on a particular training day.

Figures 1 and 2. Training Scheme. A graphical representation showing the relationship between the endurance (Figure 1) and the spring (Figure 2) training protocols. Each indicates the speed in meters per minute (X) for each training day (Y) and treadmill inclination as percent grade (Z) over the entire training period.

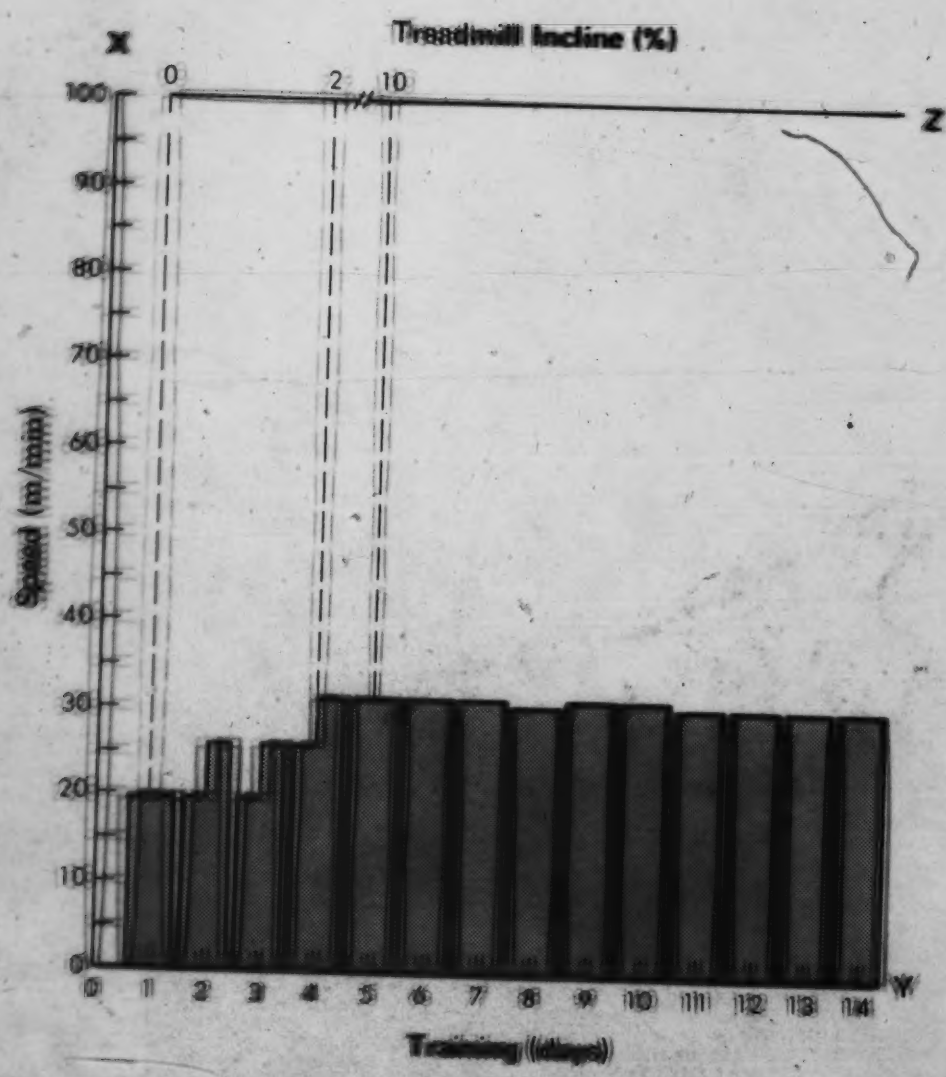


Figure 1 Endurance Training Protocol

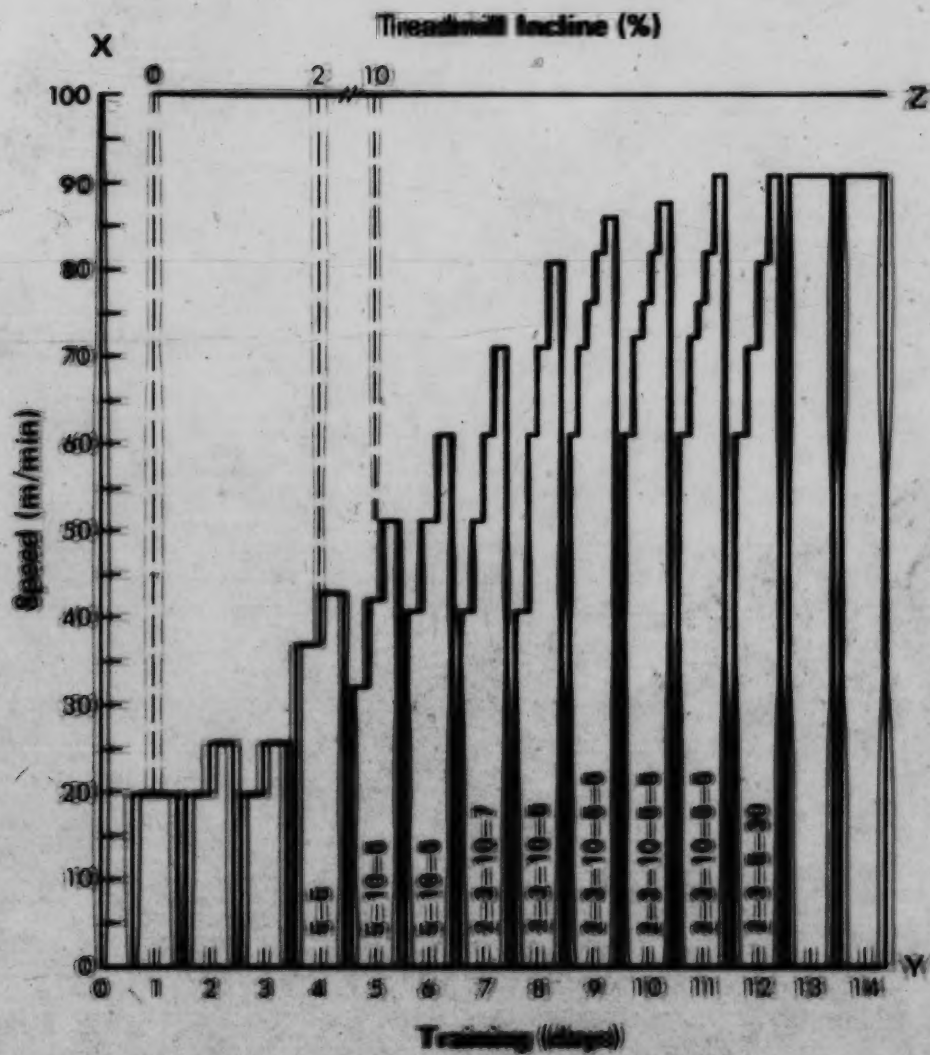


Figure 2 Sprint Training Protocol. Numbers in Columns Represent Repetitions for Each Respective Change of Speed for that Particular Group

B. Sprint Regimen

On day four, this group began running intervals consisting of 15 seconds running to 15 seconds rest. Day five saw a changed work to rest ratio of 15 seconds to 21 seconds, respectively. This work to rest ratio remained constant for all training days up to day 13. Figure 2 shows the progression of speeds for each training day with the figures in each column representing the number of repetitions for each level.

The remaining two days of the programme were designated as all animal maintenance days where the animals were running for ten seconds with a 30 second rest for 30 minutes.

C. Exercise Test

Prior to the administration of the exercise test, each of the two groups was further randomly divided into eight equal subgroups corresponding to the time of sacrifice following the exercise test. These groupings were: Pre, 0, 2, 6, 12, 18, 24 and 48 hours.

The performance test for the endurance group consisted of one hour of constant running at 30 m/min. The sprint group underwent a test of one hour and 20 minutes of intermittent running (work:rest, 10:30 seconds) at a speed of 90 m/min. Hence, the total distance covered at a 10 percent (5.75°) grade for both groups was 1800 meters.

Experimental Design

The experimental design is summarized in Table 1.

As previously mentioned, each of the endurance and sprint groups was subdivided prior to the exercise test. Subdivisions represent the time each animal was sacrificed following the test. The time period between the test and sacrifice was spent resting, i.e., no formal exercise was done. The pre-exercise group served as a basic control whereby it was sacrificed without undergoing the exercise test.

Tissue Preparation

The animals were sacrificed (8:00 a.m. to 1:30 p.m.) by decapitation with a hand operated small animal guillotine and quickly exsanguinated. The abdomen was opened and portions of the liver were excised. The tissue was lightly rinsed with physiological saline to remove hair, etc., and then immersed in isopentane cooled by liquid nitrogen. All livers were then placed in a deep freezer for storage (-70°C to -80°C) awaiting analysis.

Biochemical Procedures

Taken from: S. Seifter, S. Dayton, B. Novic and E. Muntwyler. "The Estimation of Glycogen with the Anthrone Reagent." Archives of Biochemistry, 25:191-200, 1950.

TABLE 1
EXPERIMENTAL DESIGN

[illegible]

Reagents:

1. 30 percent KOH
2. 95 percent H_2SO_4
3. 0.2 percent Anthrone Solution (0.2 g anthrone dissolved in 100 ml 95 percent H_2SO_4).
4. Working glycogen standard containing 20 mg of glucose/ml.

Procedure:

1. Weigh out 1 g of liver.
2. Place in 3 ml of 30 percent KOH solution.
3. Heat tubes in boiling water bath for 20 minutes to digest tissue.
4. Cool digest in ice, bath.
5. Transfer digest to 50 ml volumetric flask and dilute to mark with distilled H_2O .
6. Mix thoroughly.
7. Take 15 ml from this flask and place in second 50 ml volumetric flask and again dilute to the mark with distilled H_2O .
8. (a) Add 5 ml of this solution (sample) to each of two test tubes (duplicates).
(b) Add 5 ml of working glucose standard, containing a total of 100 μg of glucose, to another test tube.
(c) Add 5 ml of distilled H_2O to a fourth tube (blank).
9. Submerge all tubes in cold water.

10. Add 10 ml of anthrone reagent quickly to each tube.
11. Mix by swirling in water.
12. Remove from cold bath, cover and heat for ten minutes in boiling water bath.
13. Cool in cold water bath and read against blank at 620 nm (the colour remains stable for several hours at room temperature).

N.B. If the sample is too dark to enable a reading to be obtained, a second reading may be acquired by using two parts anthrone reagent with one part distilled H_2O .

Calculations:

$$\frac{\text{mg/ml glucose standard}}{\text{O.D. glucose standard}} = \frac{X}{\text{O.D. sample}}$$

$$X = \text{sample in mg/ml}$$

Statistical Analysis

A two way analysis of variance (DERS programme documentation: ANOV 25) for unequal n's was utilized to give a statistical analysis of the data. This was done to enable the interaction of factor A (training programme) with factor B (time of sacrifice) to be examined. A Scheffé a posteriori test was used to locate significant differences between the group means.

F values and Scheffé's contrasts were considered statistically significant if $p \leq 0.05$.

SECTION III.

RESULTS

As shown from the statistical analysis (Appendix C), there is no A main effect ($p = 0.716$). That is, when all sacrifice times are pooled, no difference was found to exist between the endurance and sprint groups.

Similarly, no interaction affect ($p = 0.787$) was found between the times of sacrifice and the endurance or sprint groups. This similarity in the glycogen repletion patterns between the two different exercise groups is displayed in Figure 3.

However, a difference was found in the B main effect ($p < 0.01$). That is, when both exercise groups were pooled, there was a significant effect across the post-exercise sacrifice times. The pattern in glycogen repletion in the pooled exercise group is illustrated in Figure 4.

The results of the mean comparisons with probability levels are listed in Appendix C. The statistically different values are summarized in Table 2.

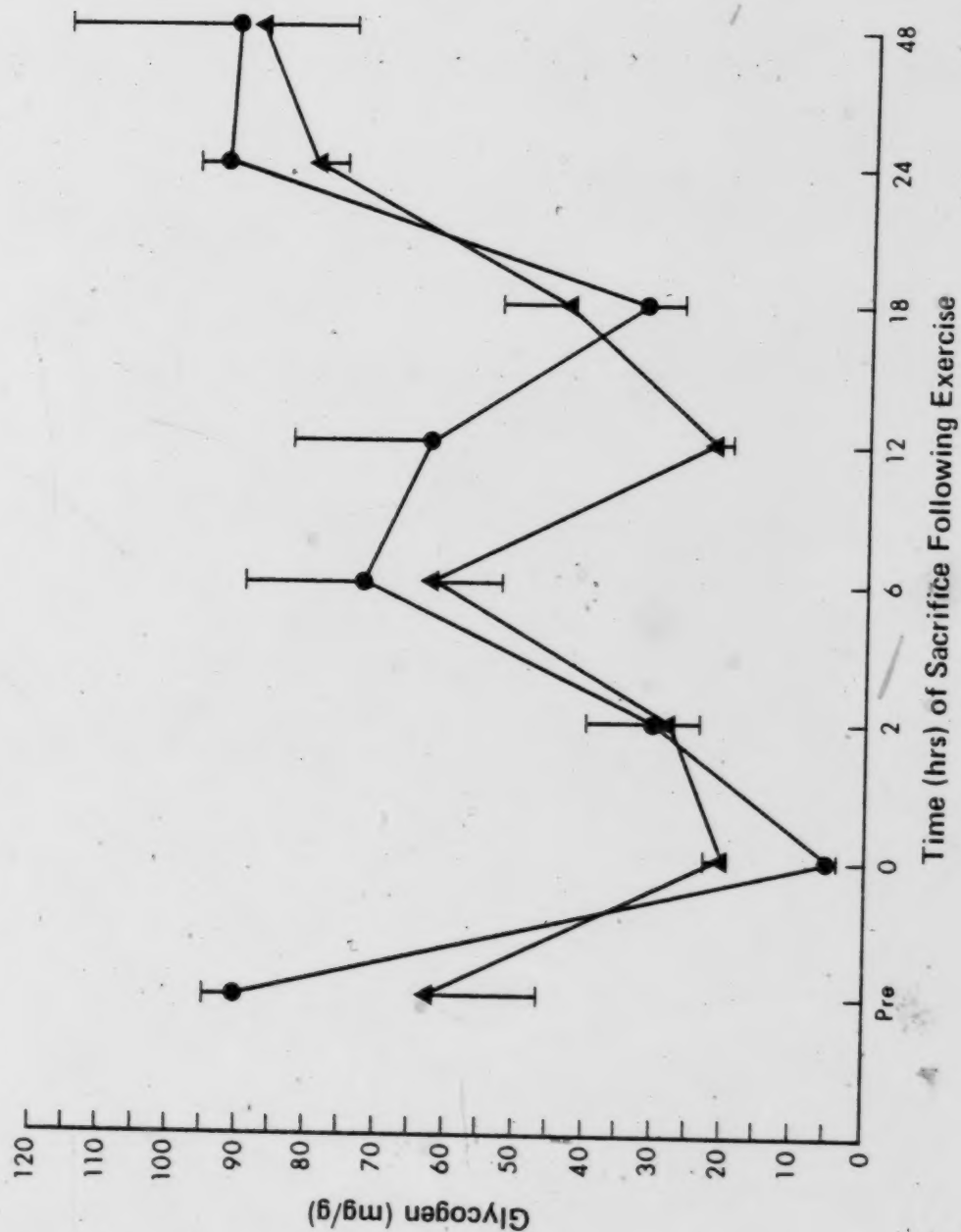


Figure 3 Cell Means of Endurance (▲) and Sprint (●) Liver Glycogens in mg/g $\pm$ SE

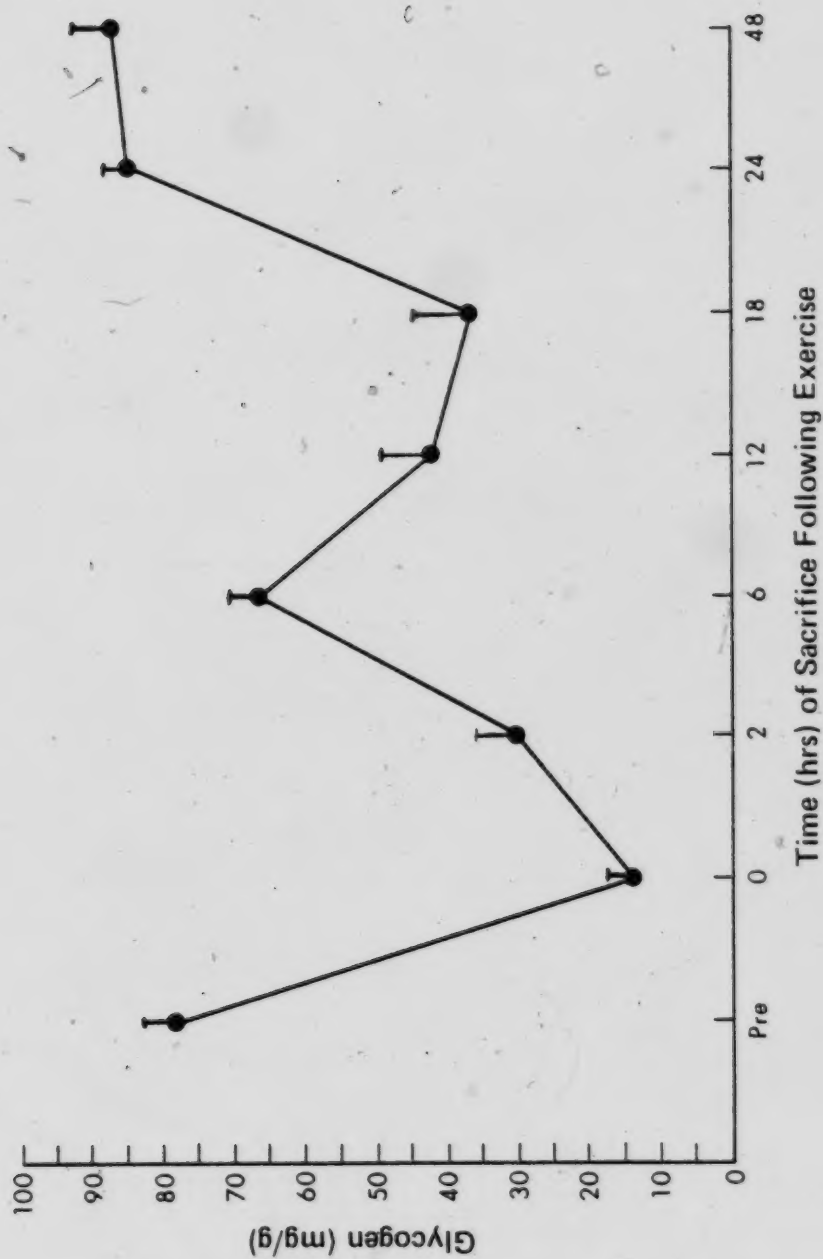


Figure 4 Alterations in Liver Glycogen Post Exercise in Combined Endurance and Sprint Groups

TABLE 2

SUMMARY OF STATISTICALLY DIFFERENT
GROUP MEANS FOR POOLED EXERCISED
GROUPS ACROSS DIFFERENT POST-
EXERCISE SACRIFICE TIMES

Pre with 0, 2, 18 hour subgroups

6 with 0

24 with 0, 2, 12, 18

48 with 0, 2, 12, 18

CHAPTER IV

DISCUSSION

Although there was no significant interaction effect, the absolute depletion of liver glycogen immediately post-exercise was 85 vs 40 mg/g for the sprint and endurance groups, respectively ($p < .01$). This large depletion by the sprint group, as compared to the endurance group, existed even though the total distance run in exercise test was the same. This is probably due to the glycogen sparing effect of aerobic exercise which utilizes an abundance of fat for energy production (Baldwin et al., 1975). However there is no difference in the pattern of liver glycogen depletion between the two exercise protocols across time (Figure 3).

Since no difference has been found between sprint and endurance groups at any of the post-exercise sacrifice times -- the two will be combined -- the resulting graph of means is shown in Figure 4.

It can be seen from Figure 4 that considerable liver glycogen depletion took place as a result of the heavy exercise test (0 hour). In this study, the glycogen replenishment occurred at six hours following heavy exercise. Armstrong and Ianuzzo (1977) found super compensation at four hours for both muscle and liver glycogen in normal and

diabetic rats. Terjung et al. (1974) found liver glycogens at 47 percent of the control level also at four hours post-exercise. Glycogen resynthesis in the muscle was found to be most rapid in the first five hours of the MacDougall et al. (1977) study. However, since no measurement was taken at four or five hours post-exercise, it would be difficult to generalize what may have occurred at these times. In fact, our values may have been higher, at four hours and then decreased to the six hour value, making all results in agreement. Similarly, most of these researchers did not test at six hours time, thus it could be possible that their values could have risen at this time. However, some variations such as these are expected. Intensity of a bout of exercise and nutritional state of the animals affects not only the degree of depletion, but also the degree and duration of the super-compensation post-exercise (Segel et al., 1975, p. 400).

In all the preceding studies, a steady, albeit slow, increase of glycogen replenishment occurred throughout the first 24 hours. This is not the case in the present study as can be seen by Figure 4. Rather, there was a marked decrease from six to 12 and 12 to 18 hours, before proceeding on an upward grade. There are two possible reasons for this occurrence, both revolving around the time which the exercise test was administered. In order to avoid diurnal variation at the time the animals were sacrificed, all animals were killed in the morning (and early afternoon) from 8:00

a.m. to 1:30 p.m. Hence, due to the nature of this study in examining specific times in the repletion pattern and to avoid wide variations in the time of the day at which sacrifice occurred, it became necessary for the animals in the 12 hour and 18 hour groups to undergo the performance tests between 10:00 p.m. (2200 h) and 3:30 p.m. (1530 h), respectively. The former time coincides with their established day (sleep) cycle and the latter at the end of the active period. The rest time is the period in which rats are known to be less active, their activity taking place during their night. Hessman (1975) examined the effect of trauma and adrenaline on muscle and liver glycogen storage. He found that while trauma decreases the storage of glycogen, it is more applicable to muscle than liver. However, infusion of adrenaline also decreases glycogen storage, especially in the liver. Increased adrenaline is concurrent with a deficiency of circulating insulin leading to a lack of glycogen storage (Hessman, 1975) since insulin plays an integral role in glycogen synthesis (Seglen, 1974). It seems to the author that by exercising the animals during the day (rest) imposes an undue stress which showed an additional increase in adrenaline over and above the level normally present during the activity period. Also, this elevated catecholamine level enhances the conversion of phosphorylase b to a and hence should result in decreased gluconeogenesis and decreased liver glycogen. The result of this exercise in the 12 hour group may therefore be a decre-

ment in the repletion seen six hours post-exercise.

Another possible explanation may lie in the 24 hour circadian rhythm for glycogen in the rat. Diurnal or circadian rhythms for glycogen levels have been demonstrated by many authors in various animals -- rabbits (Forsgren, 1928), mice (Agren et al., 1931), chickens (Sollberger, 1964), and rats (Segel et al., 1975; Sollberger, 1964; and Pessacq et al., 1975). Agren et al. (1931) showed that in spite of 40 hours of fasting in rats, an increase in liver glycogen was still observed at night, indicating that glycogen changes are, to a large extent, independent of nutritional conditions. This difference was also shown in muscle but to a lesser degree, suggesting according to Agrin, that muscle glycogen formed at night originates from the liver. Pessacq et al. (1975) substantiates this, but also indicates that muscle glycogen has identical peaks as liver glycogen, which suggests that glycogen rhythm in the intact animal is uniform regardless of the organ studied.

Glycogen exhibits a 24 hour rhythm (Segel et al., 1975; Sollberger, 1964; Pessacq et al., 1975), having a peak value two hours after the onset of the light period and a nadir two hours after the commencement of their night. Hence, the animals in the present study were given the test during the low portion of their glycogen storage cycle. Since the night is the time of maximal glycogen synthesis and is when interruption via exercise occurred, the expected elevations may be disrupted. The combination of stress and

diurnal variation may therefore be responsible for the deviation observed in the six to 12 and 12 to 18 hour groups.

Repletion to approximate pre-exercise values appears to be complete six hours post-exercise and except for the depressions at 12 and 18 hours post-exercise, the glycogen values are elevated to pre-exercise values at 24 and 48 hours also. Diet was not considered to be a confounding variable in the course of this study as food and water were provided ad libitum, and to both exercise groups.

SECTION V

CONCLUSIONS

This study examined liver glycogen repletion in sprint and endurance trained animals (rats). It has been concluded from the results presented here that:

1. Rate of glycogen depletion was greater in the sprint exercised as compared to endurance exercised animals. This occurred inspite of identical distances covered. It is suggested that the glycogen sparing effect of aerobic exercise is the major factor.
2. The two different types of acute exercise used in this study did not differentially affect liver glycogen repletion patterns.
3. Pre-exercise glycogen values were attained at 6, 24, and 48 hours following heavy exercise.
4. Variations present in the repletion pattern may be due to circadian or diurnal variations.

BIBLIOGRAPHY

- Achou, C.J. and G. Hetenyi. "Is the peripheral utilization of glucose as a regulator of hepatic glucose production of glycemia?" Canadian Journal of Physiology and Pharmacology, 52:158-165, 1974.
- Agren, G., O. Wilander and E. Jerpes. "Cyclic changes in the glycogen content of the liver and the muscles of rats and mice." Biochemistry Journal, 25:777-785, 1931.
- Armstrong, R.B. and C.D. Ianuzzo. "Exercise-induced muscle glycogen depletion and repletion in diabetic rats." Life Sciences, 20:301-308, 1977.
- Baldwin, K.M., J.S. Reitman, R.L. Terjung, W.W. Winder and J.O. Holloszy. "Substrate depletion in different types of muscle and in liver during prolonged running." American Journal of Physiology, 225(5):1045-1050, 1973.
- Baldwin, K.M., R.H. Fitts, F.W. Booth, W.W. Winder and J.O. Holloszy. "Depletion of muscle and liver glycogen during training." Pflugers Archives, 354:203-212, 1975.
- Bass, A., D. Brichzha, P. Eyer, S. Hofers and D. Pette. "Metabolic differentiation of distinct muscle types at the level of enzymatic organization." European Journal of Biochemistry, 10:198-203, 1969.
- Bergström, J. and E. Hultman. "A study of glycogen metabolism during exercise in man." Scandinavian Journal of Clinical and Laboratory Investigation, 19:218-226, 1967.
- Bergström, J., E. Hultman and A. Roch-Norlund. "Muscle glycogen synthetase in normal subjects: basal values, effect of glycogen depletion by exercise and of a carbohydrate-rich diet following exercise." Scandinavian Journal of Clinical Laboratory Investigations, 29:231-236, 1972.
- Bockman, E.L., D.K. Meyer and F.A. Purdy. "Synthesis of cardiac glycogen in relation to its' twenty-four rhythm in rats." American Journal of Physiology, 221:383-387, 1971.

- Brooks, G.A., K.E. Brauner and R.G. Cassens. "Glycogen synthesis and metabolism of lactic acid after exercise." American Journal of Physiology, 224(5): 1162-1166, 1973.
- Conlee, R.K., M.J. Rennie and W.W. Winder. "Skeletal muscle glycogen content diurnal variation and effects of fasting." American Journal of Physiology, 231(2): 614-618, 1976.
- Costill, D., A. Bennett, G. Branam and D. Eddy. "Glucose ingestion at rest and during prolonged exercise." Journal of Applied Physiology, 34(6), 1973.
- Forsgren, E. "On the relationship between the formation of bile and glycogen in the liver of rabbits." Scandinavian Journal of Physiology, 53:317, 234-240, 1928.
- Gollnick, P.D. and D.W. King. "Energy release in the muscle cell." Medicine and Science in Sports, 1(1):23-31, 1969.
- Gollnick, P.D. and L. Hermansen. Biochemical adaptations to exercise: anaerobic metabolism. In: Exercise and Sport Science Reviews, edited by J. Witmore. New York: Academic Press, 1973, pp. 1-43.
- Goodman, H. The pancreas and the regulation of metabolism. In: Medical Physiology (13th edition), edited by V. Mountcastle. St. Louis: C.V. Mosby Co., 1974, pp. 1776-1807.
- Hartley, L.H., J.W. Hogan, R.P. Jones, L.G. Kotchen, T.A. Mougey, E.H. Wherry, F.E. Pennington and L.L. Ricketts. "Multiple hormonal responses to prolonged exercise in relation to physical training." Journal of Applied Physiology, 33:607-610, 1972.
- Hessman, Y. "Glycogen storage in rat liver and skeletal muscle during continuous infusion of adrenalin." Acta Chirurgica Scandinavica, 141:393-398, 1975.
- Holloszy, J., F. Booth, W. Winder and R. Fitts. Biochemical adaptation of skeletal muscle to prolonged physical exercise. In: Metabolic Adaptation to Prolonged Physical Exercise, edited by H. Howald and J. Poortmans. Switzerland: Birkhauser Verlag Basel, 1975.
- Huston, R.L., P.C. Weisner, G.L. Dohm, E.W. Askew and J.B. Boyd. "Effects of training, exercise and diet on muscle glycolysis and liver gluconeogenesis." Life Science, 17:369-376, 1972.

- Issekutz, B., H.S. Miller and K. Rodahl. "Lipid and carbohydrate metabolism during exercise." Federal Proceedings, 25:1415-1421, 1966.
- Keul, J. "The relationship between circulation and metabolism during exercise." Medicine and Science in Sports, 5(4):209-219, 1973.
- Lamb, D.R., J.B. Peter, R.N. Jeffress and M.A. Wallas. "Glycogen hexokinase and glycogen synthetase adaptations to exercise." American Journal of Physiology, 217(6):1628-1632, 1969.
- Lamb, D.R., R. Ratcliffe and R. Kochan. "Post-exercise glycogen replenishment in untrained animals: denervation, tenotomy effects." Medicine and Science in Sports, 7(2):120-122, 1975.
- MacDougall, J.D., G.R. Ward, D.G. Sale and J.R. Sutton. "Muscle glycogen repletion after high-intensity intermittent exercise." Journal of Applied Physiology, 42(2):129-132, 1977.
- Morris, D. "Quantitative determination of carbohydrates with Dreywoods' anthrone reagent." Science, 107: 254-255, 1948.
- Pessacq, M.T. and J.J. Gagliardino. "Glycogen metabolism in muscle: its circadian and seasonal variations." Metabolism, 24(6):737-743, 1975.
- Piehl, K. "Time course for refilling glycogen stores in human muscle fibres following exercise-induced glycogen depletion." Acta Physiologie Scandinavia, 90:297-302, 1974.
- Piehl, K., S. Adollsson and K. Nazar. "Glycogen storage and glycogen synthetase activity in trained and untrained muscle in man." Acta Physiologie Scandinavia, 90:779-788, 1974.
- Ratcliffe, R.A. and D.R. Lamb. "Glycogen replenishment following exercise: effects of denervation and tenotomy." Journal of Applied Physiology, 38(6):961-964, 1975.
- Reitman, J.R., K.M. Baldwin and J.O. Holloszy. "Intra-muscular triglyceride utilization by red, white and intermediate skeletal muscle fibres of the rat." Proceedings from the Society of Experimental Biological Medicine, 142:628-631, 1973.

- Rennie, M.J., W.W. Winder and J.O. Holloszy. "A sparing effect of increase plasma fatty acids on muscle and liver glycogen content in the exercising rat." Biochemistry Journal, 156:642-655, 1976.
- Rowell, L.B. "The liver as an energy source in man during exercise." In Muscle Metabolism During Exercise, edited by B. Pernow and B. Saltine. New York: Plenum, 1971.
- Rowell, L.B. "Human cardiovascular adjustments to exercise and thermal stress." Physiological Review, 54:75-159, 1974.
- Saltin, B. "Metabolic fundamentals in exercise." Medicine and Science in Sports, 5:137-146, 1973.
- Segel, L.D., A. Chung, D. Mason and E. Amsterdam. "Cardiac glycogen in Long-Evans rats: diurnal pattern and response to exercise." American Journal of Applied Physiology, 229(2):398-401, 1975.
- Seglen, P.O. "Control of glycogen metabolism in isolated rat liver cells by glucose, insulin and glucagon." Acta Endocrinologica, 77(Suppl. 191), 1974.
- Seifter, S., S. Dayton, B. Novic and E. Muntwyler. "Estimation of glycogen with the anthrone reagent." Archives of Biochemistry, 25:191-200, 1950.
- Sollberger, A. "The control of circadian glycogen rhythms." Annals of New York Academy of Science, 117:519-522, 1964.
- Son, L.H. and E. Hultman. "Liver and muscle glycogen in man after glucose and fructose infusion." Scandinavian Journal of Clinical Laboratory Investigations, 35:5-10, 1975.
- Staudte, H.W. and D. Pette. "Correlations between enzymes of energy-supplying metabolism as a basic pattern of organization in muscle." Compositions in Biochemistry and Physiology, 41(B):533-540, 1972.
- Stryer, L. Biochemistry. San Francisco: W.H. Freeman and Co., 1975.
- Terjung, R.L., K.M. Baldwin, P.A. Mole, G.H. Klinkerfuchs and J.O. Holloszy. "Effective running to exhaustion on skeletal muscle mitochondria: a biochemical study." American Journal of Physiology, 223(3): 1972.

- Terjung, R.L., K.M. Baldwin, W.W. Winder and J.O. Holloszy. "Glycogen repletion in different types of muscle and in liver after exhausting exercise." American Journal of Physiology, 226(6):1387-1391, 1974.
- Unger, R.M. and A.M. Eisentraut. "Studies on the physiologic role of glucagon." Diabetes, 13:563-568, 1964.
- Wenger, H.A. and A.T. Reed. "Metabolic factors associated with muscular fatigue during aerobic and anaerobic work." Canadian Journal of Applied Sports Sciences, 1:43-48, 1976.
- White, A., P. Mandler and E. Smith. Principles of Biochemistry. U.S.A.: McGraw-Hill, Inc., 1973.

APPENDIX A
REVIEW OF LITERATURE

It is understood that both glycogenolysis and glucose release are closely associated during exercise to utilization of glucose by the working muscle. This is evident from the finding that, except for slight initial decrease often seen, blood glucose is maintained reasonably constant during exercise until liver glycogen stores are low (Rennie et al., 1976; Baldwin et al., 1973). The cross-circulation studies of Achou et al. (1974) have shown that the rate of tissue utilization of glucose is the regulator of the mechanism by which the hepatic glucose is released. The constancy of plasma glucose concentration is a result of near perfect equilibrium between the rates of hepatic glucose release and overall utilization. Should utilization increase, causing blood glucose to decrease (as in prolonged or repeated exercise), a compensatory increase in the rate of glucose production will re-establish normoglycemia (Achou et al., 1974; Bergstrom et al., 1967).

It is understood that during exercise, hepatic glucose production is partly under the control of the endocrine system. Alterations in liver secretions are induced by changes in blood glucose concentration and hence play a major role in blood glucose regulation (Unger et al., 1964). It is a glucose requiring hormone whose function serves to maximize liver glucose production when food is not available (Unger et al., 1964). For example, glucagon has a strong glycogenolytic effect on the liver whereas insulin acts as an inhibitor (Rennie et al., 1976; Hartley

et al., 1972).

Important to the onset of physical exhaustion in exercise is the depletion of body carbohydrates -- glucose (glycogen). One mechanism through which prolonged low intensity conditioning increases endurance seemingly implicates a glycogen-sparing effect (Baldwin et al., 1975). It would appear that the greater oxidation of fats occurs with and less from carbohydrates for aerobic energy production trained subjects than is the case with the untrained. Baldwin et al. (1975) studied the effect of aerobic training on the depletion of hepatic glycogen stores during exercise. Female rats were trained on a treadmill and by swimming. Upon completion of 14 weeks of training, exposure to three treadmill tests of 15, 30 and 45 minutes resulted in the trained animals having liver glycogens of 70.9, 61.5 and 52.6 mg/g wet weight, respectively (resting value of 72.1). In contrast, the untrained animals' values were 36.7, 21.7 and 5.3 (resting value of 42.3). As can be seen, the trained animals have lower rates of glycogen depletion throughout the test, with 28 percent decrease in liver glycogen concentration in the trained compared to 93 percent for the untrained (after 45 minutes). Terjung et al. (1972) also attained a similar glycogen depletion after an exercise test following six to 12 weeks of aerobic training. Also, as shown by the higher resting values, conditioning induces elevation of liver glycogen levels. A major advantage of this elevated glycogen (Baldwin et al.,

1975) is protection against glycogen depletion and hypoglycemia during prolonged exercise. Similar data was obtained by Rennie et al. (1976), with rats on a treadmill. Thirty minutes of exercise caused an 83 percent decrease in liver glycogen content in the control rats. The total liver glycogen depletion averaged 1,610 μ moles of glucose in the control animals and only 440 μ moles for the animals with elevated free fatty acid concentration during exercise.

Baldwin et al. (1973) examined the liver depletion of rats under different testing conditions on a treadmill. In all cases the grade was 8° but the speed and type of exercise varied (Table A.1 for results and protocol). The results showed that there was a progressive decrease in glycogen concentration. Following 160 minutes of running, depletion of liver glycogen stores were 85 percent - evidence of glucose transport into the cell followed by conversion to glucose-6-phosphate inhibiting glycogenolysis (Baldwin et al., 1973).

As to the cause of elevated glycogen levels, or the protection against glycogen depletion, recent studies have produced several theories. Rowell (1971 and 1974) has concluded that reduction of blood flow to the hepatic region during physical activity, as a training adaptation, is one possible explanation. Two other theories arise upon returning to the role of the endocrine system and hepatic glycogen. Hartley et al. (1972) found smaller increases in catecholamine levels in the trained animals during exercise and less

TABLE A.1
 RESULTS AND PROTOCOL OF STUDY BY BALDWIN et al.
 (1973) FOR LIVER GLYCOGEN

Group	Rest	15 min	60 min	120 min
1 mph; continuous run	54.57	37.95	21.20	7.31
1.5 mph; 1 minute on to 1 minute off	54.75	35.12	22.57	7.60
0.5 to 1.5 mph; ran alternate 30 second periods	54.75	50.00	19.85	5.21
values in mg/g wet weight				

reduction also in insulin levels. Since the catecholamines are glycogenolytic and insulin stimulates glycogenesis, these adaptations would enhance glycogen sparing.

As suggested by Huston et al. (1972) an increase in the activities of enzymes limiting the rate of hepatic gluconeogenesis and muscle glycolysis may be another significant adaptation to exercise, training and performance. In his study after conditioning 12 weeks, hexokinase, important in the regulation of glycogen synthesis (Piehl et al., 1974), was significantly increased by training and depressed by exhaustion.

Many researchers have examined hepatic glycogen preceding exercise. The results are generally the same, liver glycogen resynthesis is a relatively slow process (Piehl et al., 1974; Brooks et al., 1973; Lamb et al., 1969; Rennie et al., 1976). Terjung et al. (1974) found a recovery rate attaining only 47 percent of the control level after four hours post-exercise. However, the rate of glycogen synthesis was faster in the liver than in the muscles. Terjung et al. (1974) report three steps affected by exercise, which limit the conversion of glucose to glycogen: permeability of muscle to sugar, hexokinase activity and glycogen synthetase activity. They concluded that when both liver and muscle glycogen stores are depleted during exercise, repletion of liver glycogen may be the rate-limiting factor for the restoration of the capacity for prolonged strenuous exercise because hepatic repletion takes longer than mus-

cular repletion.

Ratcliffe and Lamb (1975) examined the role of the nervous system in the fairly rapid replenishment of muscle glycogen. Muscle of guinea pigs were denervated (removal of nerves) or tenotomized (removal of a portion of the Archille's tendon) following an exhaustive exercise test. It was concluded by the authors that an intact nerve supply to skeletal muscle is not essential to post-exercise glycogen replenishment. Also, diminished passive tension, or some other aspect of tenotomy, has a small but non-significant effect on glycogen refilling (Ratcliffe and Lamb, 1975).

Due to the existing knowledge of bodily dietary requirements, it is understood that repletion of glycogen stores following prolonged severe exercise depends largely upon the presence of dietary carbohydrates (Brooks et al., 1973). Following through on this concept, researchers have administered carbohydrate rich diets to their subjects on completion of heavy exercise. The end result of these efforts has been the conclusion that maximal increases in glycogen synthesis occurs with the provision of high carbohydrate diet following exercise (MacDougall et al., 1977; Bergstrom and Hultman, 1967; Costill et al., 1973; Bergstrom et al., 1972).

Presentation of the current studies discussed is given in Table A.2. Units for glycogen values vary and hence relative units are found in their respective tissue column.

TABLE A.2
 REVIEW OF CURRENT STUDIES ON GLYCOGEN REPLETION
 UNDER VARYING CONDITIONS

AUTHOR	ANIMAL	TISSUE	SPECIAL CONDITIONS	Rest.	0	2	4	8	16	24	48
Brooks et al, 1973	Rat	Muscle	Fasted 10-12 hr	360.0	60.0	115.0				150.0	
		Liver mg/100 g		1425.0	110.0	50.0				50.0	
		Muscle	Normal	490.0						310.0	
		Liver		5650.0						175.0	
Terjung et al, 1974	Rat	Red Vastus		8.3	2.0	9.8	11.3			10.7	9.0
		White Vastus		8.4	2.5	4.3	6.2			9.6	8.2
		Soleus		5.8	1.6	5.4	6.7			4.9	5.3
		Liver		42.0	0.60	13.1	19.9			52.3	11.7
		Heart		5.3	2.7	7.9	9.7			5.0	5.8
Piehl, 1974	Man	Vastus Lateralis mmoles x kg ⁻¹ wet wt.	Carbohydrates Rich Diet	125.0	21.0		65.0 *(5)	85.0 *(10)		95.0 *(22)	124.0 *(46)
Piehl, et al 1974	Man	Quadriceps Femoris mmoles x kg ⁻¹	Trained	119.0	43.0						146
			Untrained	81.0	16.0						146
			Control	4.6	3.8	4.3	3.5	4.5	4.4 *(5)	4.4	4.4
Segel, et al, 1973	Rat	Heart μg/g	Moderate trained	4.6	4.5	6.3	4.5	4.6	4.6 *(17)	5.0	
			Strenuous trained	4.6	2.8	7.4	9.0	6.5	6.5 *(17)	4.5	

*This number represents the precise time of sacrifice.

TABLE A.2 (CONTINUED)

AUTHOR	ANIMAL	TISSUE	SPECIAL CONDITIONS	HOURS SACRIFICED AFTER EXERCISE							
				Sec.	0 hr.	2 hr.	4 hr.	8 hr.	18	24	48
Armstrong & Januzzo 1977	Rat	Liver mmoles x kg ⁻¹ wet wt.	CONTROL	223.3	197.6	101.7	154.1	126.3			
			DIABETIC	86.8	81.1	58.6	87.1	91.8			
		Soleus	Control	23.8	12.0	16.9	22.1	20.6			
				34.2	15.5	24.7	30.5	23.0			
		Plantaris		31.6	13.9	25.3	27.6	22.8			
		Red Vastus		36.5	27.1	26.3	30.1	24.8			
		White Vastus		20.3	12.0	15.4	23.9	17.3			
		Soleus	Diabetic	33.0	15.7	22.2	31.1	25.3			
		Plantaris		30.2	12.6	22.3	28.8	22.7			
		Red Vastus		33.2	29.3	23.7	29.2	24.2			
Latcliffe & Lamb 1975	Guinea Pig	Gastrocnemius Muscle	Denervation	12.4							11.97
		mg/g	Tenotomy	12.4							1.51
MacDougall et al. 1977	Man	Quadriceps Muscle mmole x kg ⁻¹	Normal	80.0	25.0	30.0	50.0	59.0		80.0	
			Loaded Diet				40.0	54.0		85.0	

Biochemical Procedure

Due to the fact that liver glycogen as opposed to muscle glycogen is being examined, the direct anthrone method for glycogen determination of Seifter et al. (1950) was utilized.

The anthrone method was first adopted by Morris (1948) based upon a discovery of R. Dreywood in which he describes a specific reagent as having an extremely high specificity for carbohydrates. He also suggests it's value in quantitative determinations of such substances. According to Seifter et al. (1950), the mechanism of the reaction between anthrone and carbohydrates was studied by Sattler and Zerban (1938) where the sulfuric acid medium of the reagent causes dehydration of the sugar to a furfural derivative (an aromatic compound). This derivative then condenses with anthrone forming a blue coloured compound.

Because of the quick aging of the reagent, Morris (1948) stresses that a known glucose standard be included with each series of unknowns.

Morris (1948) also explains why 620 nm is best for reading the samples. This reading (as opposed to 540 nm) gives a higher sensitivity and decreases any errors caused by extraneous colours.

APPENDIX B
LIVER GLYCOGENS

TABLE B.1
 RAW DATA OF LIVER GLYCOGENS (mg/g) OF ENDURANCE AND SPRINT TRAINED RATS AT
 VARYING TIMES FOLLOWING TWO DIFFERENT EXERCISE RESTS

	Time (hr)							
	Pre	0	2	6	12	18	24	48
Endurance	51.4	19.3	25.5	53.7	23.9	53.3	76.3	64.8
	30.2	22.1	23.1	69.2	23.5	29.9	84.0	104.7
	86.2	--	34.9	--	18.1	44.9	--	95.5
	86.6	--	--	--	--	--	--	--
Sprint	92.3	5.9	36.9	82.5	83.6	28.0	87.3	73.6
	85.1	5.0	23.4	58.8	31.0	24.0	91.4	108.3
	97.1	4.3	--	--	73.9	43.1	98.2	--

TABLE B.2
CELL MEANS mg/g + SE OF LIVER GLYCOGENS AT VARIOUS INTERVALS
FOLLOWING TWO DIFFERENT EXERCISE RESTS

	Pre	Time (hr) Sacrificed						
		0	2	6	12	18	24	48
Endurance	63.6	20.7	27.8	61.4	21.8	42.7	80.1	88.0
	+16.0	+1.4	+4.4	+11.0	+2.3	+8.4	+5.4	+14.7
Sprint	91.5	5.1	30.1	70.7	62.8	31.7	92.3	91.0
	+4.3	+0.6	+9.5	+16.8	+19.9	+7.1	+3.9	+24.5

APPENDIX C
STATISTICAL ANALYSIS

TABLE C.1
ANALYSIS OF VARIANCE: TEST FOR ADDITIVITY

Source	Sum of Squares	D.F.	Mean Squares	F Ratio	Probability
SAB	0.357519E+04	7	0.510741E+03	2.097020	0.078676
SE	0.657600E+04	27	0.243556E+03		

N.B. In order to obtain a more accurate error estimate, one pools the SAB and SE; therefore the test with no interaction is more conservative when testing main effects.

TABLE C.2
ANALYSIS OF VARIANCE TABLE FOR LIVER GYCOGENS OF TRAINED RATS

Source	Sum of Squares	D.F.	Mean Squares	F. Ratio	Probability
SA	0.103283E+04	1	0.103283E+04	3.459317	0.071567
SB	0.307566E+05	7	0.439380E+04	14.716448	0.000001
SE	0.101512E+05	34	0.298564E+03		

TABLE C:3
SCHEFFE'S MULTIPLE COMPARISONS OF MAIN EFFECTS

I	J	Constant	F Ratio	Probability
1	2	65.92	6.02	.00013
1	3	46.51	3.02	.01408
1	4	10.21	0.13	.99574
1	5	33.94	1.78	.12431
1	6	39.07	2.36	.04485
1	7	-10.18	0.14	.99377
1	8	-13.91	0.27	.96150
2	3	-19.40	0.45	.86583
2	4	-55.70	3.29	.00882
2	5	-31.98	1.33	.26600
2	6	-26.85	0.94	.49034
2	7	-76.01	6.93	.00004
2	8	-79.82	7.55	.00002
3	4	-36.30	1.39	.23816
3	5	-12.58	0.21	.98182
3	6	- 7.45	0.07	.99930
3	7	-56.70	3.81	.00372
3	8	-60.41	4.37	.00152
4	5	23.72	0.65	.71500
4	6	-28.85	0.96	.47821
4	7	-20.40	0.44	.86889
4	8	-24.12	0.62	.73776
5	6	5.13	0.04	.99992
5	7	-44.11	2.53	.03280
5	8	-47.84	2.98	.01509
6	7	-49.25	3.16	.01110
6	8	-52.97	3.65	.00482
7	8	- 3.72	0.02	.99999

APPENDIX D

LABORATORY CHOW CONSTITUENTS

PURINA LABORATORY CHOW CONSTITUENTS

Crude protein, not less than.....	23.0%
Crude fat, not less than.....	4.5%
Crude fibre, not less than.....	6.0%
Ash, not more than.....	9.0%

Fish meal, meat and bone meal, wheat gum meal, animal liver meal, dried skim milk, dried beet pulp, ground extruded corn, ground oat groats, soybean meal, dehydrated alfalfa meal, can molasses, vitamin B₁₂ supplement, vitamin A supplement, vitamin E supplement, animal fat preserved with BHA, calcium pantothenate, chlorine chloride, folic acid, riboflavin supplement, brewer's dried yeast, thiamin, niacin, D activated plant sterol, calcium carbonate, dicalcium phosphate, iodized salt, iron sulphate, iron oxide, magnesium oxide, cobalt carbonate, copper oxide, zinc oxide.

